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Development of benzoyl peroxide loaded nanosponges gel

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Abstract
Objective: Benzoyl peroxide (BPO) is a prominent ingredient in topical acne treatments. The goal of this study was to synthesize Benzoyl peroxide (BPO) nanosponges using various polymers and were designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and modify drug release profiles. This system can work efficiently for systemic as well as local effect.
Methods: Nanosponges were prepared by quasi emulsion solvent diffusion method using different polymers. The best performing nanosponges were integrated into the gel formulation. Prepared gel formulations were evaluated for physical parameters like viscosity, pH, and clarity and *in-vitro* drug permeation study.
Result: The drug release data of selected batch were fitted into different kinetic models which show that the drug release from gel formulations follows zero order release. Selected gel formulation batch was compared with the marketed formulation.
Conclusions: Thus it can be concluded that nanosponge delivery system work efficiently for topical delivery of Benzoyl Peroxide.

Keywords: Nanosponge, topical gel, benzoyl peroxide, antimicrobial activity

Introduction
Benzoyl Peroxide (BPO) is commonly used in topical formulations for the treatment of acne and, more recently, athlete's foot. The use of BPO has advantages in comparison to the use of antibiotics because potential bacterial resistance is avoided, and it is also preferred over keratolytic agents due to its bactericidal effect [1]. Skin dryness, peeling, and transitory local edoema may occur, and contact sensitization has been described in some individuals who have used benzoyl peroxide-containing treatments. The degree of irritation is thought to be related to the amount of BPO present in the skin, and Benzoyl Peroxide encapsulation can significantly lessen side effects [2]. The controlled release of drug from the formulation into the epidermis such that the drug remains primarily localized with only a restricted amount entering the systemic circulation, is a means of controlling side-effects [3]. Nanosponges are porous polymeric delivery systems that are small spherical particles with large porous surface. Nanosponges can significantly reduce the irritation of drugs without reducing their efficacy. As compared to other nanoparticles, nanosponges are insoluble in water and organic solvents, porous, nontoxic and stable at high temperatures up to 300^0 C. The size of the nanosponges ranges from 250nm-1µm in diameter. Nanosponges are formed as three-dimensional networks of spherical porous particles having colloidal sizes with a mean diameter of less than 1µm and narrow size distribution and form opalescent suspensions when dispersed in water [4]. The goal of this study was to design BPO nanosponges with various polymers and drugs: Polymer ratios were determined using a quasi-emulsion solvent diffusion method, and the effect of drug: polymer ratio, polymer: solvent ratio, as well as the emulsifier concentrations and stirring rate on the physical properties of the nanosponges, and compare the release rate.

Material and Method
Benzoyl Peroxide was gifted from the Luxica Pharma, Bharuch, and Gujarat, India. Other ingredients like HPMC K4M, Eudragit S100, Eudragit RL100 dichloromethane, Ethyl Cellulose and Poly Vinyl Alcohol was procured from Loba Chem, Vadodara, and Gujarat, India.

Preparation of Benzoyl Peroxide Nanosponge

Nanosponge were prepared by quasi-emulsion solvent diffusion method using an external phase of distilled water and polyvinyl alcohol (PVA), and internal phase consisting of drug, ethyl alcohol, polymer and dichloromethane as a solvent for ethyl cellulose (EC) and polymer. For preparing Nanosponge, the internal phase was prepared and added to the external phase at room temperature. After emulsification process is completed, the mixture was continuously stirred for 2 hours. Then the Nanosponges were separated by filtration. The product was washed and dried by vacuum oven at 40°C for 12 hrs. HPMC and euadrit and were characterized [5].

Evaluation of Benzoyl Peroxide Nanosponge

Particle size: Particle size was measured using Malvon Zetasizer Nano S. These data were recorded in table 2.

Production yield: The production yield of the nanoparticles was determined using following equation [6, 7].

$$\text{Production yield} = \frac{\text{Practical mass of nanosponges}}{\text{Theoretical mass (polymer + drug)}} \text{ X } 100$$

Loading efficiency: The drug content of nanosponges was determined spectrophotometrically (λ max = 232nm). A sample of Benzoyl Peroxide nanosponges (100mg) was dissolved in 100ml of phosphate buffer (pH 6.8) and kept it for overnight. The drug content was calculated and expressed as actual drug content in nanosponge. The loading efficiency (%) of the nanosponges was calculated according to the following equation [6, 7]:

$$\text{Loading efficiency} = \frac{\text{Actual drug content in nanosponge}}{\text{Theoretical drug content}} \text{ X } 100$$

Preparation of Benzoyl Peroxide nanosponge gel

Accurately weighed amount of Carbopol 934 was taken and sprinkled on water with stirring using propeller. Nanosponge formulation containing Benzoyl Peroxide was added to the above solution with constant stirring. This final solution was neutralized slowly by adding tri-ethanolamine with constant stirring until the gel is formed [8, 9].

Evaluation of Nanosponge gel

Nanosponge gel formulations were evaluated parameters like viscosity, pH, clarity, drug content, *in-vitro* drug permeation and *In-vitro* release kinetics. Physical parameter study of nanosponge formulation is given in table [10].

***In-vitro* permeation study**

Modified Franz diffusion cell was used for these studies. Full thickness abdominal skin of male wistar albino rats weighing 140 to 200g was used for the skin permeation and the deposition studies**.** Briefly, to obtain skin, animals were sacrificed. Hair from the abdominal region was carefully removed and an excision in the skin was made. The dermal side of the skin was thoroughly cleaned of any adhering tissues. Dermis part of the skin was wiped 3 to 4 times with a wet cotton swab soaked in isopropanol to remove any adhering fat. The skin specimens were cut into appropriate size after carefully removing subcutaneous fat and were washing with normal saline. Skin was mounted in a modified Franz diffusion cell, kept at 37ºC. The known quantity (1gm gel containing 10mg of the drug) was spread uniformly on the skin on donor side. pH 6.8 phosphate buffer was used as the acceptor medium, from which samples were collected at regular intervals during 12 hours and replaced with the same amount of buffer to maintain the receptor phase at 22ml or 25ml and J *flux* of Benzoyl Peroxide was calculated and reported in table [11].

Viscosity: Viscosity of prepared gels was measured by Brookfield Viscometer. Apparent viscosity was measured at 25°C and rotating the spindle at 10rpm [10].

pH: The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter [10].

Clarity: It was determined visually by using clarity chamber.

Drug Content: Gel formulation containing Benzoyl Peroxide equivalent to (100mg) was dissolved in methanol, filtered and the volume was made to 100ml with methanol. The drug content was determined by diluting the resulting solution for 10 times with methanol and measuring the absorbance at 232nm using Shimadzu-1700 UV Visible spectrophotometer [10].

Anti-microbial testing: All of the prepared formulations were checked for their antimicrobial activity against *Staphylococcus aureus,* one of the bacteria responsible for causing acne, using the cylinder cup method. The Cylinder cup method is the most widely used method to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. This method relies on the inhibition of bacterial growth measured under standard conditions. For the purpose of this test, a culture medium, specifically the Mueller-Hinton agar medium, is uniformly and aseptically inoculated with the test organism. Ditch the bore in plate. Add compounds solution in bore. To check the antimicrobial activity of the prepared formulations, a concentration of 100mg/ml of all of the prepared formulations was prepared. The concentrations of each formulation were incorporated into the bore. Later, the disc of each formulation was placed into the agar medium for 24 hours at 37°C. It gave a clear zone of inhibition around the bore, indicating the antimicrobial activity of the formulations. The zones of inhibition so obtained were measured and the results were compared [12].

Stability study: Stability study of Nanosponge gel was performed for 60 days as per ICH guidelines at 40^0 C $\pm$ 0.5^0C and 75 $\pm$ 5% RH (relative humidity). The samples were taken at the interval of 20, 40 and 60 days [13].

Kinetic modelling: In order to understand the kinetics and mechanism of drug release, the results of *in-vitro* drug release were fitted into various kinetic equations like zero order (cumulative% release vs. time), first order (log% drug remaining vs. time), Higuchi's model (cumulative% drug release vs. square root of time), Korsmeyer peppas plot (log of cumulative% drug release vs. log time). R2 (coefficient of correlation) and n (Diffusion exponent) values were calculated for the linear curve obtained by regression analysis of the *in-vitro* drug permeation plots [14].

Results

Benzoyl Peroxide nanosponge

Nanosponge were prepared by quasi-emulsion solvent diffusion technique with distilled water containing polyvinyl alcohol (PVA) as an external phase, and internal phase consisting of drug, ethyl alcohol, polymer and dichloromethane as a solvent for EC and polymer. The production yield of Benzoyl Peroxide nanosponge formulation is shown in the table below.

Table 1: Formulation of Benzoyl Peroxide nanosponge

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	1100	1100	1100	1100	1100	1100	1100	1100	1100
	Internal phase								
HPMC K4-M	100	200	300	-	-	-	-	-	-
Eudragit RL-100	-	-	-	100	200	300	-	-	-
Ethyl Cellulose	-	-	-	-	-	-	100	200	300
Di-chloro methane	20	20	20	20	20	20	20	20	20
	External phase								
PVA	100	100	100	100	100	100	300	300	300
water(ml)	150	150	150	150	150	150	150	150	150
% yield	42.5 ± 2.45	49.6 ± 0.9	27.5 ± 1.2	37.5 ± 1.34	45.6 ± 0.78	23.14 ± 0.91	77.5 ± 0.45	78.6 ± 2.86	82.1 ± 2.31

*All quantities are in mg; *each observation is the mean ± s.d. of three determinations

The batches from F1-F6 containing polymer HPMC and Eudragit did not give good yield (less than 50%) to produce nanosponge and hence were not included in further study. Three batches, Batch 7 to batch 9, containing ethyl cellulose were sleeted as they were showing promising results for production yield.

Particle size and loading efficiency for three batches, batch 7 to batch 9, were performed and reported in the table below.

Table 2: Characterization of Benzoyl Peroxide nanosponge

Formulation	Particle size(diameter)	Loading efficiency (%)
F7	135-239nm	67.22±2.37
F8	100-213nm	53.33±0.56
F9	80-200 nm	87.9±1.38

The above data revealed that loading efficiency (87.9 ± 1.38%) and particle size (80-200nm) was found better in F9 formulation in comparison to other formulations. So F9 batch was entrapped into gel and evaluated further.

Nanosponge gel

Three different nanosponge gels were prepared by taking 1%, 3% and 5% of drug loaded nanosponge in carbopol 934 gel base containing propylene glycol, Tri-ethanolamine was used to neutralize the pH.

Table 3: Characterization of Benzoyl Peroxide nanosponge

Ingredients	A	B	C
Drug loaded nanosponge	1% w/w	3% w/w	5% w/w
Carbopol 934	30 mg	30 mg	30 mg
Propylene glycol	0.5 mg	0.5 mg	0.5 mg
Tri ethanolamine	q.s	q.s	q.s
Water	up to 100g	up to 100g	up to 100g
Viscosity (cps)	3285	3353	3462
pH	6.8	7.1	6.9

All three gel formulations demonstrated desired viscosity (3200-3500cps) and pH values (6.8 – 7.2). All the three gel formulations were subjected to *in-vitro* dissolution study to check amount of drug release over a period of time and release pattern with kinetic modelling.

Table 3: *In-vitro* release profile of drug from nanosponge gel formulations

Time in Hrs	Batch F9A	Batch F9B	Batch F9C
1	2.71	1.15	0.83
2	5.92	5.11	3.76
3	9.54	9.42	9.5
4	13.58	11.11	18
5	16.54	21.16	21
6	23.49	25	30
7	28.98	40	37.43
8	34.96	50.23	47.8
9	41.29	57.81	65
10	48.57	69.9	73.33
11	56.05	77.38	84
12	63.66	84.99	98.39
J Flux (mg/cm2 h)	0.07691	0.02379	0.1672

Table 3 indicated better release of drug in gel formulation batch F9C as compared to other two. From the data of *in-vitro* permeation study expressed as J flux, it can be interpreted that nanosponge loaded gel formulation having better permeability than the pure drug. So it can be concluded that nanosponge formulation enhanced the permeability of drug in current study as the J flux values increased with increase in amount for drug loaded nanosponge.

Antimicrobial testing

The results were obtained for formulation F9A, F9B and F9C showed 5.5mm, 11.3mm and 17.4mm zone of inhibition respectively, which correlates that as the amount of drug loaded nanosponge increased in gel from 1% to 5%, the antimicrobial activity also increased.

Fig.1 Zone of inhibition of F9A

Fig.2 Zone of inhibition of F9B

Fig.3 Zone of inhibition of F9C

Fig.4 Solvent effect

Comparison of selected Benzoyl Peroxide Nanosponge Gel with marketed formulation

Selected Benzoyl Peroxide Nanosponge gel formulation F9C was compared with marketed formulation (MF) (BENIDE GEL containing 5% Benzoyl Peroxide) and the data was recorded in a table below.

Table 4: Comparison of Physical Parameters

Formulation	Viscosity (cps)	pH	Clarity	Drug content (%)
F9C	3462	6.5	Clear and transparent	95.74
MF	3321	6.8	Clear and transparent	97.73

***In-vitro* drug permeation**

In-vitro diffusion profile of F9C and MF Drug permeation data of F9C and MF are shown in table**.** The total amount of drug permeated was found to be 98.3% and 92% after a period of 9 and 12 hrs for MF and F9C respectively.

Table 5: Diffusion profile of MF (Marketed formulation) and F9C

Time in Hr	MF	F9C
1	4.60	0.83
2	11.46	3.76
3	19.30	9.5
4	28.16	18
5	37.81	21
6	49.55	30
7	62.83	37.43
8	76.86	47.8
9	91.81	65
10	---	73.33
11	---	84
12	---	98.39

Kinetic modelling

In order to understand the kinetics and mechanism of drug release, the results of *in-vitro* drug release were fitted into various kinetic equations like zero order (cumulative% release *vs.* time), first order (log% drug remaining *vs.* time), Higuchi's model (cumulative% drug release *vs*. square root of time), Korsemeyer peppas plot (log of cumulative% drug release *vs*. log time). *R*2 (coefficient of correlation) and *n* (Diffusion exponent) values were calculated for the linear curve obtained by regression analysis of the *in-vitro* drug permeation plots. The release study data of Benzoyl Peroxide loaded nanosponge gel formulation F9C and marketed formulation (MF) was analysed using rate constant equation such as zero order, first order, Higuchi and Korsemeyer-Pappas equation showed that F9C formulation had the tendency to follow zero order diffusion pattern of drug permeation, whereas MF had first order diffusion pattern for drug permeation. Drug transport mechanism was found to be Non fickian diffusion.

Table 6: Kinetic modelling data

Formulation	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas model	
				(r^2)	n value
F-9C	0.9755	0.8771	0.9755	0.9989	0.6444
MF	0.9562	0.9119	0.9562	0.9888	0.2266

Stability study

The selected nanosponge gel was stable for at least 60 days at 40 ± 0.5^{0}C temperature and 75 ± 5% RH, with negligible change in viscosity and drug permeation in 12 hrs. The nanosponge gel formulation revealed that the formulation has sufficient stability.

Table 7: Stability study data

Formulation	Viscosity			% Drug permeated in 12 hrs		
F9C	20 days	40 days	60 days	Initial	30 days	60 days
	3415	3361	3254	98.39	97.7	96.79

Similarity factor (stability study data) revealed that there was no significant difference in the release and permeation rate of the formulation before and after stability studies so the formulation is consider to be stable.

Discussion

The prepared Benzoyl Peroxide formulation was formulated into gel, which controlled the release for up to 12 hours. The final Benzoyl Peroxide gel was then compared with marketed Benzoyl Peroxide gel. When the prepared gel was compared to the marketed gel, it was reported that the drug release kinetics were of zero order, i.e. controlled release, whereas the marketed BENIDE GEL has release kinetics of first order. Release kinetics of selected nanosponge gel formulation correspond best to zero order release model and drug release mechanism as per *n* value of Korsmeyer-Peppas was found to be 0.64 which indicated Non-fickian zero order release. After stability study no physical change were observed. Hence, the formulation was stable and prepared Nanosponge was found to be less irritating and also shows good antimicrobial activity.

Conclusions

Benzoyl Peroxide nanosponge gel was formulated using different polymers such as HPMC, Euadragit and EC with solvents such as polyvinyl alcohol and water and the study conclusively demonstrated that Benzoyl Peroxide can be successfully encapsulated into Nanosponge by quasi emulsion solvent diffusion using Ethyl cellulose as polymer.

Abbreviations

HPMC-Hydroxy Propyl Methyl Cellulose
EC-Ethyl Cellulose
MF-Marketed Formulation
PVA-Poly Vinyl Alcohol
BPO-Benzoyl Peroxide

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